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## Analysis of the exine micromorphology of *Epipactis helleborine* (L.) CRANTZ and *Epipactis albensis* NOVÁKOVÁ et RYDLO (Orchidaceae: Neottieae) and its application to genus taxonomy\*

ANNA JAKUBSKA

Department of Biodiversity and Plant Cover Protection, Institute of Plant Biology,  
University of Wrocław, Kanonia 6/8, 50-328 Wrocław, e-mail: Ajak@biol.uni.wroc.pl

**ABSTRACT.** The *Epipactis* ZINN, 1757 genus belongs to the so-called ‘critical’ and controversial plant taxa. The aim of the research was to determine whether the analysis of micromorphology of the pollen grains (particularly the exine structure) is useful in identification of two species, i.e. *Epipactis helleborine* (L.) CRANTZ and *Epipactis albensis* NOVÁKOVÁ et RYDLO, which are difficult to distinguish between. No substantial differences in the exine ornamentation or the pollen size of the two species, which would allow to identify the species or confirm the diagnosis in doubtful cases have been found in the course of the research.

**Key words:** pollen, exine micromorphology, *Epipactis helleborine* (L.) CRANTZ, *Epipactis albensis* NOVÁKOVÁ et RYDLO, orchids

Orchidaceae family is well known for wide diversity of its pollen in regard to its micromorphology (i.e. HUYNH 1977, ACKERMAN & WILLIAMS 1980, ZAVADA 1990, Dressler 1993, SCHILL & PFEIFFER 1997). This diversity is noticeable at many levels, e.g. the variability of arrangement of the pollen grains in pollinia or the sculpturing of the exine. These qualities are successfully used in the taxonomy of Orchidaceae (LUMAGA et al. 2006).

The taxonomic value of a feature or a group of features is variable and depends on the taxonomic group, which makes it impossible to predict the value of such a feature beforehand without examining it carefully (STACE 1993).

Comparative morphology of pollen grains gives us knowledge about their taxonomic position and can indicate the separate character of a taxon or its wrong classification.

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The aim of the research was to examine whether studying the microstructure of pollen grains can help identify the species of *Epipactis* ZINN, 1757 genus, particularly the critical taxa.

The presented results are of preliminary character and a part of a project on exine microstructure of the genus *Epipactis* ZINN, 1757.

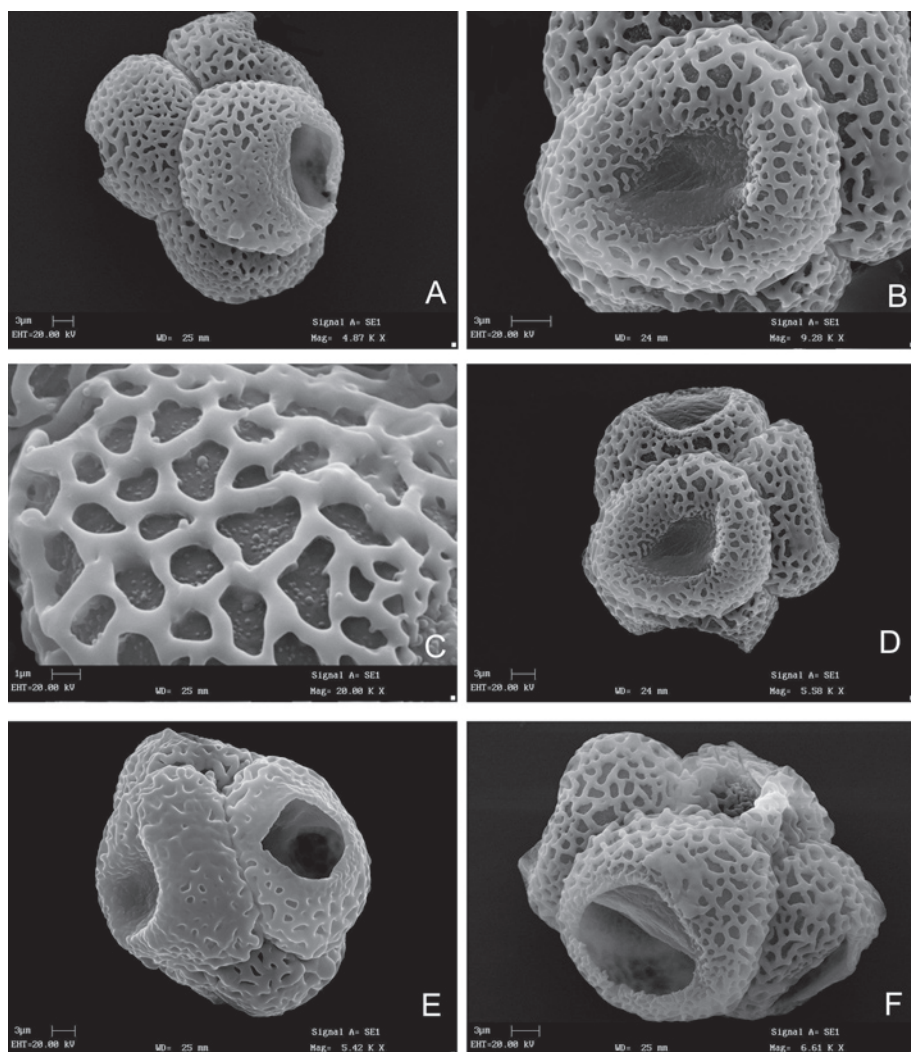


Plate 1. The sculpturing of exine of the pollen grains in taxa of the genus *Epipactis* ZINN, 1757: A – Tetrad pollen with a visible reticular surface (*exinum reticulatum*) in *Epipactis albensis* NOVÁKOVÁ et RYDLO, B, C, D – Details of the reticular surface of *E. helleborine* (L.) CRANTZ pollen grains, E, F – Undeveloped (E) and deformed (F) tetrad pollen of *Epipactis albensis* NOVÁKOVÁ et RYDLO

## MATERIALS AND METHODS

## PLANT MATERIAL

Pollinaria were collected from plants growing in Poland and the Czech Republic and from herbarium materials. To cover intraspecific variation, pollinaria of 25 individuals per species were sampled. The pollen used in the slides was collected from buds as well as from open flowers. Biometric measurements, e.g. the length of the polar axis and the diameters of openings of reticulum, were carried out in order to compare the size of individual pollen grains.

## SCANNING ELECTRON MICROSCOPY (SEM)

Scanning microscope LEO 435 VP produced by LEO (Zeiss+Leica) company was used in the research together with an apparatus for freezing slides in liquid nitrogen „Cryo” CT 1500 Oxford <sup>TM</sup>. The slides were prepared with the use of the freezing technique (Cryofixation). Pollinaria were fixed in FAA (formalin-acetic acid-alcohol 10:5:50), dehydrated in an ethanol series, critical-point dried in liquid CO<sub>2</sub> and sputter-coated with approx. 30 nm of gold.

## RESULTS

The pollen of the studied species of *Epipactis* ZINN, 1757 genus is spread in the form of tetrads with characteristic exine sculpturing (Plate 1). No substantial differences between the studied species in the micromorphology of the exine surface or in the size of the pollen grains, which would allow to identify the taxa were found in the course of the examination. It may result from the young age of the species. The exine sculpturing in both species is of the same type, i.e. reticular surface (*exinum reticulatum*). In the case of *Epipactis albensis* pollen, 18 % of the grains were undeveloped, smaller or deformed (Plate 1E, 1F). In the case of *E. helleborine*, the defective pollen accounted for approximately 4 %, although the figure varies depending on the quality of the material under examination. The external diameter of the pollen is 20,25-26 µm in *E. helleborine* and 20,18-25,6 µm in *E. albensis*, yet the average figures are approximate.

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